Bullinularia foissneri nov. sp. (Amoebozoa, Arcellinida) from Australia and synopsis of the genus *Bullinularia**

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Abstract: *Bullinularia foissneri* nov. sp. was collected in soil samples from Mt. Buffalo National Park Victoria, Australia. The new species has a very conspicuous test morphology. The tests of this large species (143–207 µm) is round to egg-shaped, the aperture is close to the front end and lies at the bottom of a deep furrow that separates the apex and the dorsal apertural lip from the bellied ventral side. By these characters *B. foissneri* can be distinguished from all other species of the genus easily. A synopsis of the genus shows that *B. pulchella* Schönborn, 1964 is almost identical to *B. minor* Hoogenraad & De Groot, 1948 and should be seen as a synonym. *Bullinularia navicula* Bonnet, 1979 has no pores around the aperture and is transferred to *Plagiopyxis*.

Key words: Humus, new synonymy, *Plagiopyxis navicula* nov. comb., scanning electron microscopy, taxonomy.

Introduction

The genus *Bullinularia* Deflandre, 1953 (for *Bullinula* Penard, 1911) comprises medium to large lobose testate amoebae with an agglutinate test. These are built from mineral particles in a sheet-like organic cement. All species have an eccentric, invaginated (cryptostome) pseudostome where the aperture of the shell is completely or partially hidden by the dorsal apertural lip. A key character are the pores on the dorsal lip, on the apex and, depending on the species, on the ventral side. In conventional classifications (e.g. Meisterfeld 2002) *Bullinularia* is placed together with genera like *Plagiopyxis*, *Geoplagiopyxis*, *Paracentropyxis*, *Protoplagiopyxis*, *Hoogenraadia* and *Planhoogenraadia* within the family Plagiopyxidae. Based on a first partial SSU rRNA sequence *Bullinularia indica* branches in all trees within the Arcellinida (Nikolaev et al. 2005).

Bullinularia is one of the smaller genera of testate amoebae. Until now seven species have been described from mosses and soils. Here I present the description of a new, very conspicuous species from Australia. In this context I will discuss a synopsis of all *Bullinularia* species, their classification and nomenclatural consequences.

Material and methods

Mount Buffalo National Park comprises a granite massif separated from the western side of the Great Dividing Range in north-eastern Victoria (Australia). The granite massif rises sharply from the surrounding plain, including granite boulders, outcrops and monoliths, massive bluffs and near-vertical granite rock faces which soar a thousand metres above the Ovens River valley. The Mount Buffalo plateau is supporting a range of vegetation types, including subalpine heaths, herb fields, grasslands and subalpine woodlands. Its highest elevation is the Horn (1723 m). The Park has a high annual rainfall, with maxima of over 2500 mm at higher elevations. Snow contributes significantly to total precipitation above 1200 m. The Park is 337 km by road north-east of Melbourne, south of the Ovens Highway between Myrtleford and Porepunkah.

Samples were taken in the winter of 1997 at Mt. Buffalo N. P. Victoria, Australia. The sampling site was below Mt. Dunn and the samples consist of humus directly beneath Mountain Gum trees (*Eucalyptus dalrympleana*).

The material had been collected in self-sealing plastic bags, kept at a constant temperature of 18°C, later transferred to screw-capped 10-ml plastic centrifuge tubes and preserved in formalin.

* This species is dedicated to Wilhelm Foissner (Salzburg) on the occasion of his 60th birthday.

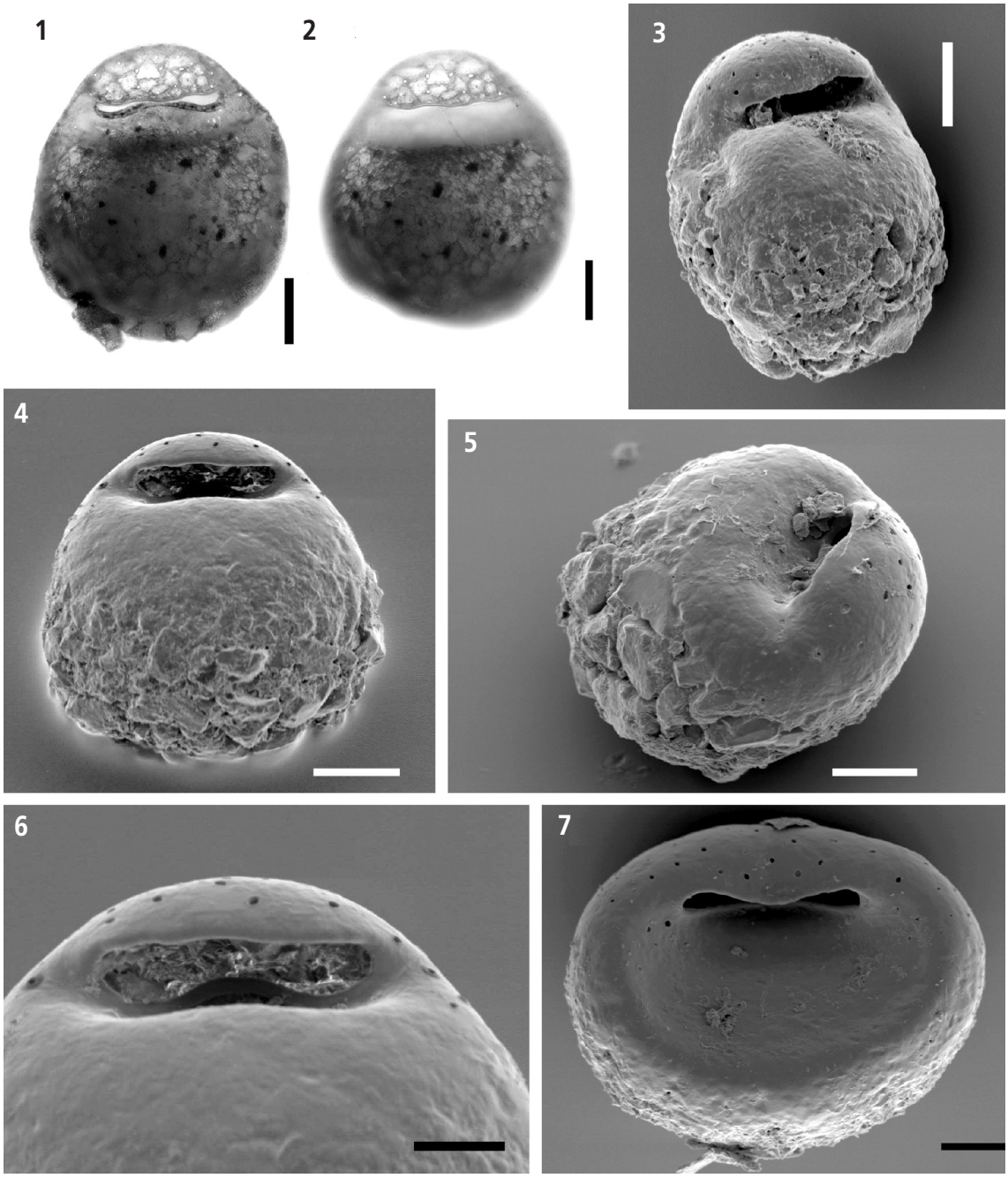

Fig. 1–7: Light (**1, 2**) and scanning electron micrographs (**3–7**) of *Bullinularia foissneri* (**3–6**) and *B. indica* (**7**). **1**: Ventral view, combined stack of six images. Note the rim of the ventral apertural lip. **2**: Ventral view. The focus lies on the dorsal lip (high numerical aperture 0.85!). The aperture and the ventral lip are out of focus. **3**: Slender specimen. Note the deep furrow. The organic cement covers only the anterior half of the belly and the dorsal lip. **4**: Broad specimen. **5**: Frontal view showing the bellied ventral face. **6**: Apertural view showing the deep furrow and the thickened rim of the ventral apertural lip. Usually this lip is prolonged. In almost all specimens the dorsal lip does not hide the pseudostome. **7**: Ventral view. Scale bars: 40 µm (1–5, 7), 20 µm (6).

A complete species list of all testate amoebae found in Mt. Buffalo N.P. can be found in MEISTERFELD & TAN (1998).

Stacks of light microscopical images were combined with the freeware program CombinZM (http://hadleyweb.pwp.blueyonder.co.uk/CZM). Specimens for scanning electron microscopy (SEM) were dehydrate in an alcohol series and manually mounted on carbon adhesive conducting tabs (PLANO), sputter-coated and observed with a Cambridge S600 SEM.

Description of *Bullinularia foissneri* nov. sp.

Differential diagnosis: *Bullinularia foissneri* differs from all congeners in the following features:

The apertural furrow is very conspicuous.

The ventral face is bellied.

The l/b ratio is between 0.97 and 1.40.

Type locality: Humus from below Mt. Dunn in the Mt. Buffalo National Park in Victoria, Australia.

Type material: A syntype preparation is deposited in the collection of the Oberösterreichische Landesmuseum (Biologiezentrum) Linz (LI), Upper Austria.

Morphology (Tab. 1): The shape of this species is variable. The test is in ventral view oval or egg-shaped, rarely round, the ventral face is always more or less bellied! The aperture is close to the front end and lies at the bottom of a deep furrow (Fig. 2, 6). The ventral apertural lip is steeply invaginated, its forward edge is curved and thickened (Fig. 1, 6). The dorsal lip only partly cowers the aperture. The edge is either bent forward or straight. Pores are always present, easily visible as brighter spots (Fig. 1, 2) but restricted to the dorsal lip and the apex of the test. In contrast to *B. indica* (Fig. 7) they never occur on the belly of the shell. In light microscopical observation at higher apertures the lateral margins of the apertural furrow are out of focus (Fig. 2).

In lateral view the tests are high (Fig. 5), they sometimes resemble certain *Hoogenraadia* species, for example *H. cryptostoma* GAUTHIER-LIÈVRE & THOMAS, 1958.

The test is composed of a mixture of mineral particles of different size. On the ventral face these elements are smaller. The overall colour of the shells is light brownish but at a closer view one finds a mosaic of almost transparent as well as completely dark or brown particles (Fig. 1, 2). The cement between the mineral particles is dark. The surface of the test around the pseudostome and the apex is covered by smooth organic cement (Fig. 5) all other parts are more or less rough due to larger grains of sand.

The nucleus is large (about 35 µm across), from the ovular type with numerous small nucleoli.

Geographical distribution: Until now *B. foissneri* is only known from the type location, but HOOGENRAAD & DE GROOT (1948) have figured in their work on the testate amoebae of New Zealand an egg-shaped *Bullinularia* which they considered to be a monstrous form of *B. indica*. Their Fig. 16 resembles *B. foissneri* and this could be a hint that the new species is also present in New Zealand. A comparison with almost all published images of *Bullinularia* shows that a comparable *Bullinularia* had never been figured and this makes it likely that *B. foissneri* is an endemic of the Australis. After *B. indica* and *B. gracilis* it is the third species known from Australia.

Synopsis and discussion of the genus *Bullinularia*

The variability within the genus *Bullinularia* is relatively large and follows different trends. Although all species built an agglutinate test, the fractions of organic material and mineral particles as well as the colour of the organic cement vary considerably. As a consequence, the tests can be almost opaque or transparent and dark brown to colourless. The shape of the test varies between the egg-shaped *B. foissneri* (Fig. 3, 4) and the broad oval *B. navicula* (Fig. 13) and also the dimensions are very different. The breadth of *Bullinularia* species ranges from 70 µm (*B. minor*) up to 256 µm (*B. indica* in JUNG 1934). All species fall into three partly overlapping size classes (Tab. 2).

Bullinularia foissneri, *B. indica* (Fig. 7) and *B. lithophora* (Fig. 9) form the largest class. Some overlap between this group and the next one exists. *Bullinularia foissneri* has a very conspicuous shape (Fig. 1, 3–5) and can be distinguished from all other species easily. The same is true for *B. lithophora* (aperture, composition of the test, colour) but the classification is problematic because BONNET (1974) in his diagnosis did not mention pores around the pseudostome; in addition, the original

Tab. 1: Dimensions of the test of *Bullinularia foissneri* nov. sp.[1]

Character	Min	Max	Mean	SD	n
Length	143	207	185	17.0	24
Breadth	141	200	165	19.6	24
l/b ratio	0.97	1.40	1.13	0.13	24
Aperture	61	86	71	8.4	24
Height	108	161	138	12.7	24

[1] All measurements in µm. b – breadth, l – length, Max – maximum, Mean – arithmetic average, Min – minimum, n – number of specimens investigated, SD – standard deviation.

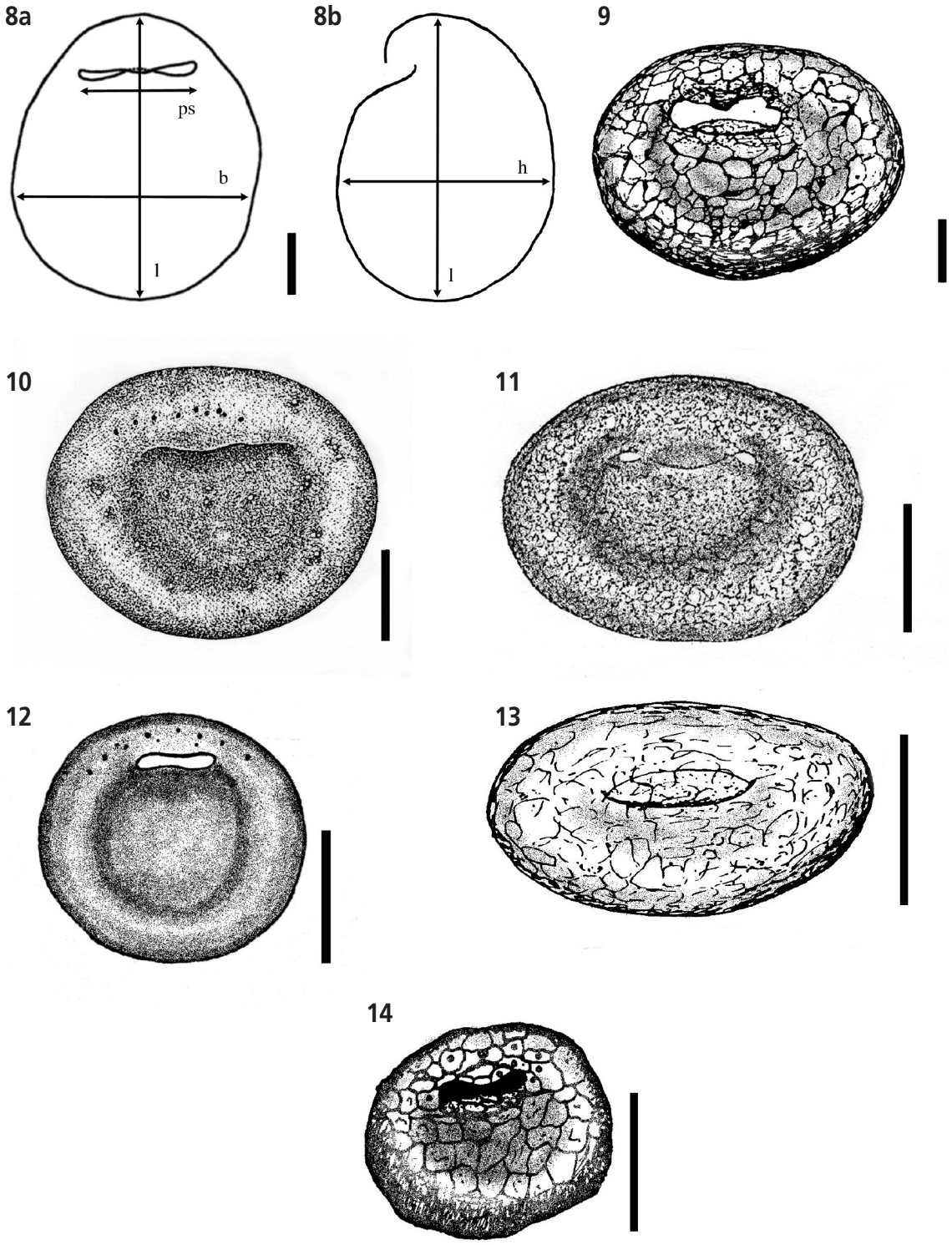

Fig. 8–14: Drawings of different species of *Bullinularia*. **8**: *B. foissneri* ideal individual (a, ventral view; b, lateral view). **9**: *B. lithophora* (after BONNET 1974). **10**: *B. devexa* (after COÛTEAUX & MUNSCH 1978). **11**: *B. gracilis* (after BONNET & THOMAS 1960). **12**: *B. minor* (after HOOGENRAAD & DE GROOT 1948). **13**: *B. navicula* (after BONNET 1979). **14**: *B. pulchella* (after SCHÖNBORN 1964). Scale bars: 40 µm. b – breadth, h – height, l – length, ps – pseudostome breadth.

drawings do not show them doubtlessly. Therefore the position within the family Plagiopyxidae remains uncertain until new information is available. *Bullinularia indica* has been reported frequently from mosses (very often *Sphagnum*), the other species prefer humus and soils.

An intermediate size group is formed by *B. devexa* COÛTEAUX & MUNSCH, 1978 (Fig. 10), *B. gracilis* THOMAS, 1959 (Fig. 11), and a small form of *B. indica*. The classification of these taxa is not very clear.

A nomenclatural problem is the status of the small forms of *B. indica*. PENARD (1911: 226) had mentioned smaller *B. indica* shells (130–170 µm). He stated that these forms are connected with the type by numerous transitions. In 1912 PENARD states that in some locations the smaller form replaces the type completely while in other places where they occur together they are not connected by any transitions (PENARD 1912: 9). He also mentioned a third very broad form that probably corresponds to *B. gracilis*. PENARD explicitly did not describe a new form or variety of *B. indica*! Nevertheless BARTOS (1940, 1954) and others have used the name *B. indica minor* with PENARD as author. Formally *B. indica minor* is a nomen nudum.

Morphological differences between *B. gracilis* and tests of the small forms of *B. indica* are vague or not very significant. *Bullinularia gracilis* is usually slightly broader with a l/b ratio between 0.70 and 0.86 (but see LÜFTENEGGER & FOISSNER 1991) while some populations of the small *B. indica* have almost circular tests. BARTOS (1963) considered *B. gracilis* even as a synonym of the small *B. minor* (HOOGENRAAD & DE GROOT, 1948). Subsequent authors (GOLEMANSKY 1968; VUCETICH 1976; LÜFTENEGGER & FOISSNER 1991) have extended the size range to 110–192 µm that there is an almost complete overlap in size between the two and *B. devexa*. Also the range of the l/b ratio varies continuously between the three taxa (Tab. 2). Morphologically *B. gracilis* and *B. devexa* as well as the small *B. indica* can be seen as the extremes of a continuum.

A database analysis shows that in contrast to the small *B. indica* which has been found mostly in *Sphagnum* or acid humus *B. devexa* and *B. gracilis* seem to prefer neutral or calcareous substrates (e.g., THOMAS 1959, BONNET & THOMAS 1960, BONNET & COMOY 1977, BONNET 1993; COÛTEAUX & MUNSCH 1978) but the true ecological requirements of the taxa are obscured by the uncertain classification.

The smallest group consists of *B. minor* (HOOGENRAAD & DE GROOT, 1948) (Fig. 12), *B. navicula* BONNET, 1979 (Fig. 13) and *B. pulchella* SCHÖNBORN, 1964 (Fig. 14). The size of *B. pulchella* falls into the range of

Tab. 2: Synopsis of all *Bullinularia* species (data mainly from the original descriptions). [1] From drawings.

Species	***B. foissneri* nov. sp.**	***B. devexa***	***B. gracilis***	***B. indica***	***B . indica* (small form)**	***B. lithophora***	***B. minor***	***B. navicula***	***B. pulchella***
Author	MEISTERFELD (2008)	COÛTEAUX & MUNSCH (1978)	THOMAS (1959)	PENARD (1907)	?	BONNET (1974)	HOOGENRAAD & DE GROOT (1948)	BONNET (1979)	SCHÖNBORN (1964)
Length (µm)	143–207	97–149	–	–	–	$\bar{x}$ = 166, s = 21	70–83	51	–
Breadth (µm)	141–200	124–169	median 120	170–200 (250)	130–170	$\bar{x}$ = 199, s = 21	77–102	88	70–72
Length/breadth	0.97–1.40	0.78–1.00	0.70–0.86[1]	0.78–1.00	as type	about 0.83	0.81–0.90	0.55–0.60	0.84–0.95
Height	108–161 µm	82 µm[1]	53 µm[1]	45–60 % of breah	as type	–	30–50 % of diameter	51 µm	45 µm
Apertural furrow	yes	no	no	no	no	no	no	no	no
Pores	yes	yes	yes	yes	yes	?	yes	no	yes
Colour	brownish	dark brown, opaque	chamois to brownish, transparent	brown to dark brown, opaque	as type	more trans - parent than *B. indica*	colourless or yellowish, transparent	colourless or yellowish, transparent	brown
Ventral side	+/- bellied	flat, depressed	flat, depressed	flat, depressed	flat, depressed	flat, depressed	flat, depressed	flat, depressed	flat, depressed
Nucleus	about 35 µm, ovular	unknown	unknown	45 µm, vesicular with peripheral chromatin (PENARD 1912; Fig. 4)	unknown	unknown	unknown	unknown	unknown
Biotope	litter, soil	litter, soil Mangroves, calcareous bedrock	soil and moss on calcareous substrates, basophile and aerophile	moss, *Sphagnum*, acid soils	as type	soil	moss	forest soils	soil
Distribution	Victoria (Australia)	Guadeloupe	cosmopolitan	cosmopolitan	cosmopolitan?	Tropics: Indonesia, Ivory Coast, Nepal, Paraguay	cosmopolitan?	Tropics: Angola, Gabon, Ivory Coast, Nepal, Thailand, Venezuela	Germany, Romania, Russia

B. minor. Except minor differences in colour there are no distinct characters that separate the two species. Therefore *B. pulchella* is considered as a synonym of *B. minor*! In *B. navicula* the longitudinal axis (anterior to posterior) is much smaller than the breadth (Fig. 13). Problematic is the lack of pores on the dorsal lip. These pores are the most important character that separates *Bullinularia* from *Protoplagiopyxis* or *Plagiopyxis*! To maintain the concept of the genus *Bullinularia* it is necessary to transfer *Bullinularia navicula* BONNET, 1979 to *Plagiopyxis*: *P. navicula* (BONNET, 1979) nov. comb.

Outlook

The status of the several *Bullinularia* species and the relation of the genus to other members of the Plygiopyxidae remains open to discussion. With morphological and biometrical methods we will not be able to resolve the inter- and intraspecific relations and it will not be possible to decide whether the observed adaptive peaks are genetically isolated units and deserve the status of a species or are simply morphs of a size-polymorphic "species". Only a combination of thorough morphological and molecular methods will us allow to define boundaries between species and genera more objectively, to estimate the true global diversity and to describe the geographical distribution correctly.

A short synonymy and nomenclature of the genus *Bullinularia*

***Bullinularia* DEFLANDRE, 1953**

1953 *Bullinularia* DEFLANDRE, Traite de Zoologie **I**: 127, 128, Fig. 90A–E (replacement name)

Type species (by monotypy): *Bulinella indica* PENARD, 1907

Remark: Although *Bulinella* PENARD, 1907 is a valid name it has not been in use during the last 90 years and for reasons of stability the name *Bullinularia* DEFLANDRE, 1953 should be kept.

Species included in *Bullinularia*:

***Bullinularia devexa* COÛTEAUX & MUNSCH, 1978**

1978 *Bullinularia devexa* **COÛTEAUX & MUNSCH**, Rev. Ecol. Biol. Sol **15**: 396, Pl. I, Fig. 1, Pl. II, Fig. 2, 4

***Bullinularia foissneri* nov. sp.**

2008 *Bullinularia foissneri* this paper

***Bullinularia gracilis* THOMAS, 1959**

1959 *Bullinularia gracilis* THOMAS, Procès.Verbaux. Soc. Linn. Bordeaux **97**: 37, Pl. 9–10

***Bullinularia indica* (PENARD, 1907) DEFLANDRE, 1953**

1907 *Bulinella indica* PENARD, J. Roy. Micros. Soc. **1907**: 274–277, Pl. 14, Fig. 1–4

1911 *Bullinula indica* PENARD – PENARD, Brit. Antarct. Exped. Biol. **1**: 225–226 (unjustified replacement name; PENARD mistakenly thought that *Bulinella* was a preoccupied name for a mollusc, but *Bulinella* is an incorrect spelling of *Bullinella* NEWTON, 1891; by replacing the correct name he created a homonym of *Bullinula* SOWERBY, 1839).

1912 *Bullinula indica* PENARD – PENARD, Rev. Suisse Zool. **20**: 1–9, Pl. 1, Fig. 1–5

1940 *Bullinula indica* var. *minor* PENARD – BARTOS, Arch. Protistenk. **94**: 153 (nomen nudum)

1953 *Bullinularia indica* (PENARD 1907) – DEFLANDRE, Traité de Zoologie **I**: 127, 128, Fig. 90A–E (combination with *Bullinularia*)

1954 *Bullinula indica minor* PENARD, 1911 – BARTOS, Korenonozce Radu Testacea, Bratislava: 67 (nomen nudum)

***Bullinularia lithophora* BONNET, 1974**

1974 *Bullinularia lithophora* – BONNET, Bull. Soc. Hist. Nat. Toulouse **110**: 283, Fig. 1, 2

***Bullinularia minor* (HOOGENRAAD & DE GROOT, 1948) BARTOS, 1963**

1948 *Bullinula minor* HOOGENRAAD & DE GROOT, Hydrobiol. **1**: 41–43, Fig. 17

1963 *Bullinularia minor* HOOGENRAAD & DE GROOT 1948 – BARTOS, Acta Soc. Zool. Bohemoslov. **27**: 88 (new combination)

1964 *Bullinularia pulchella* – SCHÖNBORN, Limnologica **2**: 106, Pl. 1, Fig. 11 (new synonym)

Species misplaced in *Bullinularia*:

***Plagiopyxis navicula* (BONNET,** 1979) nov. comb.

1979 *Bullinularia navicula* BONNET, Bull. Soc. Hist. Nat. Toulouse **115:** 106, Fig. A 1-3

Remark: For foundation of transfer, see above.

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